



Nutritional Composition of Edible Oysters (*Crassostrea madrasensis* L.) from the Southwest Coast of India

Kajal Chakraborty, Selsa J. Chakkalakal, Deepu Joseph, and Minju Joy

Marine Biotechnology Division, Central Marine Fisheries Research Institute, Cochin, Kerala, India

ABSTRACT

Nutritional composition of edible oysters (*Crassostrea madrasensis*) from the wild and cultured growth habitats from the southwest coast of India were evaluated over 4 years (2008–2011) during the premonsoon season. The important nutritional qualities of this species have been correlated with chlorophyll-*a* concentration, sea surface temperature, and phytoplankton density in their growth environments. The higher proportions of total polyunsaturated fatty acids, eicosapentaenoic, and docosahexaenoic acids in the edible oysters collected from the wild habitats were significantly correlated with chlorophyll-*a* concentration revealing the role of the phytoplanktons to contribute to the occurrence of these vital fatty acids. The ideal atherogenic index (AI); thrombogenicity index (TI); hypocholesterolemic/hypercholesterolemic ratio (HH); and balanced quantities of vitamins, minerals, amino acids, and low cholesterol contents qualified *C. madrasensis* as a potential health food.

KEYWORDS

edible oyster; *Crassostrea madrasensis*; nutrients; fatty acids; amino acids; climatic factors

Introduction

Oysters are one of the most valued seafoods as they constitute a rich source of fatty acids, amino acids, and minerals, which are essential for providing a balanced diet (Nagabhushanam and Bidarkar, 1978). Bivalves in coastal areas are an excellent source of *n*-3 polyunsaturated fatty acids (PUFAs), including the long-chained eicosapentaenoic acid (20:5*n*-3; EPA) and docosahexaenoic acid (22:6*n*-3; DHA; Taylor and Savage, 2006). The consumption of these bivalve mollusks also provides an inexpensive source of protein with a high biological value, essential minerals, and vitamins (Astorga España et al., 2005). The edible oyster, *Crassostrea madrasensis*, is a bivalve mollusk (family *Ostreidae*); found in the coastal sea beds in the Arabian Gulf in the south Malabar waters along the west coast of Peninsular India, and its farming is becoming increasingly popular (Mallia et al., 2009). The oyster fishery of Kerala is limited to backwaters like Dharmodam, Kayamkulam, Ashtamudi, Paravoor, and Kadalundi where *C. madrasensis* is the most prominent species, but the culture operations are possible only for 6 months during the premonsoon period (Harikumar and Rajendran, 2007).

Considering the promising perspective for the utilization of *C. madrasensis* as a potential health food, studies on its biochemical composition have begun to receive considerable attention. This work anticipated the influence of the growth conditions on the essential nutritional compositions of *C. madrasensis* harvested from the southwestern coast of India. No reports have yet been published about the nutritional composition of this species under wild and cultured conditions. The present study directed to elucidate the effect of various climatological parameters on the nutritional profile of *C. madrasensis* throughout the study period (2008–2011). The data might provide useful information for seafood industries and edible oyster aquaculture. The co-relation with the nutritional composition and climatological factors was attempted to focus for a more holistic assessment of the overall biological significance of wild and cultured *C. madrasensis*.

Materials and methods

Materials

The samples (wild and cultured) were collected during the months of April and May (premonsoon season) in 4 consecutive years (2008–2011) from the intertidal rocky shore of Sattar Island at Cochin, situated at the southwest coast of India (Lat: 9° 58' N; Long: 76° 16' E; Figures 1A–C), which is predominantly influenced by oceanic water from the Arabian Sea. The samples were collected from comparable depths (2 m below the surface) to avoid possible interference of variations in depth on the nutritional composition. Cultured oysters were stripped from the 0.25-inch rope at each site. All samples, after cleaning of fouling organisms, were immediately transported to the laboratory in an icebox.

Determination of biometric parameters and condition indices

The samples of *C. madrasensis* were measured for their biometrical parameters—namely, length, width, and thickness. Length (maximum measure along the anterior-posterior axis), width (maximum lateral axis), and thickness (depth of maximum longitudinal axis) of 30 randomly selected samples were measured using a vernier caliper. The samples were then weighed, opened by cutting the adductor muscle with a scalpel, and the wet meat and shell weight were noted (Figure 1D). The dry weight was determined by oven drying of the wet tissues for 48 h at 60°C. Condition indices (CI) were used to characterize the apparent health and quality of the biological entity. Three condition indices taken into account in this study are as follows: (a) Economic CI (Imai and Sakai, 1961) as $\text{thickness} \times [0.5 (\text{length} + \text{width})]^{-1}$; (b) Booth CI (Booth, 1983) as $\text{wet flesh weight} \times \text{total weight}^{-1}$; (c) Ecophysiological CI (Walne, 1976) as $\text{dry flesh weight/dry shell weight}$. The dry weight of the shells was determined by oven-drying at 80°C.

Biochemical analysis

The edible muscle parts of the samples of *C. madrasensis* were separated manually by cutting the adductor muscle as stated earlier. The entire amount of pooled edible portion (edible flesh) was

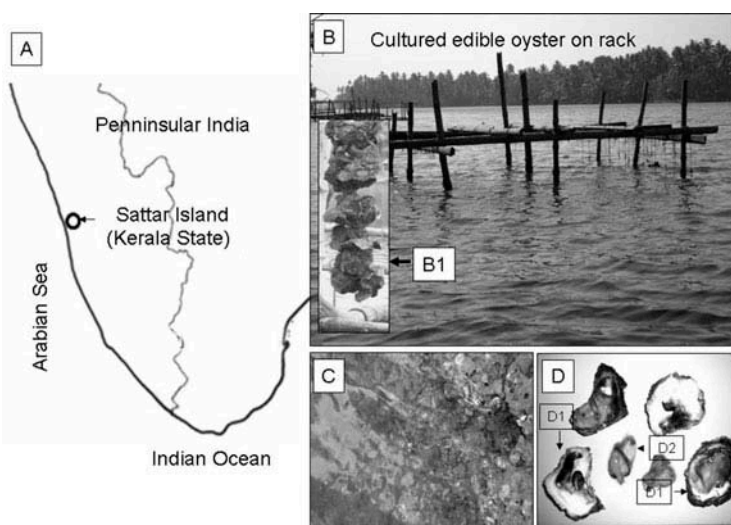


Figure 1. Sample collection site of edible oyster (*C. madrasensis*, wild and cultured) along the southwestern coast of India. Sattar Island at Cochin (UST: 1141160 E, 221200 N; Lat: 9° 58' N, Long: 76° 16' E) was selected as the collection sites of *C. madrasensis* (A); a site of culture of *C. madrasensis* by the rack method (B); cultured *C. madrasensis* on rack (B1); wild *C. madrasensis* (C); *C. madrasensis* (D); shell with meat (D1) and meat portion (D2) of *C. madrasensis*.

thereafter ground in a mincer and packed in insulated containers at -20°C before being used for biochemical analysis with respect to fatty acid, total cholesterol, amino acid, vitamin, and mineral composition.

Estimation of lipids and fatty acid profiling

The extractions of the lipids in the tissues of wild and cultured oysters were carried out by the Folch extraction method (Folch et al., 1957) using chloroform:methanol (2:1, v/v; 200 mL). The extracted lipids were determined gravimetrically in triplicate. The fatty acid composition of the total lipids from *C. madrasensis* was determined as described elsewhere (Metcalf et al., 1966). The gas-liquid chromatography (GLC) conditions (HP 5890 Series II; Perkin Elmer, Shelton, CT, USA) were as reported earlier (Chakraborty et al., 2014). Nitrogen (ultra high purity > 99.99%) was used as carrier gas at 25 cm/s flow rate. Hydrogen was used as the carrier gas at a head pressure of 20 psi. The injection volume was 0.02 μL . Fatty acid methyl esters (FAMES) were identified by comparison of retention times with known standards (SupelcoTM 37 Component FAME Mix, Catalog No. 47885-U; Supelco Inc., Bellefonte, PA, USA), and the results were expressed as percent weight of total fatty acids (%TFA). The different ratios of fatty acid indicating nutritional values of the edible oysters—viz., *n*-3/*n*-6, *n*-6/*n*-3, DHA/EPA, PUFA/saturated fatty acid (SFA), and linoleic acid (LA)/ α -linolenic acid (ALA) were calculated. In order to characterize the atherogenic and thrombogenic potential of the oyster meat, the indices of atherogenicity (AI) and thrombogenicity (TI) have been calculated using the equations of Ulbricht and Southgate (1991) and Barrento et al. (2010), respectively, as follows: $\text{AI} = (12:0 + 4 * 14:0 + 16:0)/(\text{MUFA} + n\text{-3 PUFA} + n\text{-6 PUFA})$; $\text{TI} = (14:0 + 16:0 + 18:0)/[(0.5 * \text{MUFA}) + (0.5 * n\text{-6 PUFA}) + (3 * n\text{-3 PUFA}) + (n\text{-3 PUFA}/n\text{-6 PUFA})]$. These indices are considered as the cardiovascular disease risk factors. The hypocholesterolemic/hypercholesterolemic (HH) ratio was determined using the method described by Santos-Silva et al. (2002), where $\text{HH} = (18:1n\text{-9} + 18:2n\text{-6} + 20:4n\text{-6} + 18:3n\text{-3} + 20:5n\text{-3} + 22:5n\text{-3} + 22:6n\text{-3})/(14:0 + 16:0)$. The HH ratio considers specific effects of fatty acids on cholesterol metabolism, and high HH values are desired from a nutritional point of view.

Total cholesterol content

The total cholesterol content in the edible portion of oysters was determined spectrophotometrically (Varian, Cary, NC, USA), as described elsewhere (Wanasundara and Shahidi, 1999) with suitable modification using o-phthalaldehyde (50 mg dL^{-1} in glacial acetic acid). The total cholesterol content of the samples was calculated from the standard curve of cholesterol and expressed as mg/100 g edible portion (mg/100 g EP).

Estimation of amino acids

Amino acid contents of the edible portion of oysters were measured using Pico-Tag method as described earlier (Heinrikson and Meredith, 1984) using suitable modifications. The samples (0.1 g) were hydrolyzed with HCl (6 N, 10 mL) at 110°C in sealed glass tubes for 24 h on a multiplace heating mantle. The aliquot containing hydrolyzed amino acids was treated with redrying reagent (methanol 95%:water:triethylamine, 2:2:1 v/v/v), and thereafter precolumn derivatization of the amino acids was performed with phenyl isothiocyanate (PITC, or Edman's reagent) to form phenylthiocarbamyl (PTC) amino acids. The reagent was freshly prepared, and the composition of derivatizing reagent comprised of methanol 95%:triethylamine:phenylisothiocyanate (20 μL , 7:1:1 v/v/v, 70 μL methanol + 10 μL distilled water + 10 μL triethylamine + 10 μL phenyl isothiocyanate). The derivatized sample (PTC derivative, 20 μL) was diluted with sample diluent (20 μL , 5 mM sodium phosphate NaHPO_4 buffer, pH 7.4: acetonitrile 95:5 v/v) before being injected into reversed-phase binary gradient high performance liquid chromatography (HPLC; Waters Corporation,

Milford, MA, USA) fitted with a column maintained at $38 \pm 1^\circ\text{C}$ in a column oven to be detected by their UV absorbance (λ_{max} 254 nm). The mobile phase comprised of (A) sodium acetate trihydrate (0.14 M, 940 mL, pH 6.4) containing triethylamine (0.05%), mixed with acetonitrile (60 mL) and (B) acetonitrile:water (60:40, v/v). A gradient elution program, with increasing eluent B, was employed for this purpose. The quantification of amino acids was carried out by comparing the peak area of sample with the standard, and the amino acid content was expressed as g/100 g protein.

Estimation of vitamins

Estimation of fat soluble vitamins was carried out by a modified method of Salo-Vaananen et al. (2000). The stock solutions of vitamin standards (Sigma-Aldrich Chemical Co. Inc., St. Louis, MO, USA) were prepared (1, 10, 25, 50, and 100 ppm) to draw the standard curve by HPLC. All the stock solutions were stored at -20°C , except vitamin D₃ where the stock solutions were stored at 4°C . Aliquots of the lipid (0.1 g with 0.02% T-BHQ, w/w) were hydrolyzed with KOH/MeOH (0.5 N, 2 mL) at 30°C for 30 min. The hydrolyzed mixture (2 mL) was extracted with petroleum ether (12 mL) and washed with 25 mL of distilled water (2×8 mL) to make it alkali free. The nonsaponifiable matter (8 mL) was concentrated using a rotary evaporator (50°C), reconstituted in HPLC MeOH, filtered through nylon acrodisc syringe filter (0.2 μm) and injected (20 μL) in HPLC (Shimadzu LC 20AD, Shimadzu Corp., Nakagyo-ku, Japan) equipped with a C18 column (Phenomenex, 250 mm length, 4.6 mm I.D., 5 μm) in column oven (32°C) and connected to a photo diode array (PDA) detector. The run time was 45 min, the eluents were detected at 265 nm, and the flow rate was 1 mL/min. Vitamin C was determined based upon the quantitative discoloration of 2,6-dichlorophenol indophenol titrimetric method as described elsewhere (AOAC, 1995). The vitamins A, D₃, E, and C were expressed as IU/100 g EP and vitamin K₁ as $\mu\text{g}/100$ g EP.

Estimation of minerals

Estimation of minerals was carried out by atomic absorption spectrophotometer by the di-acid ($\text{HNO}_3/\text{HClO}_4$) digestion method as reported earlier (Astorga España et al., 2005), with suitable modifications. In brief, the wet tissues (20 g) were dried in oven at 105°C to obtain a constant weight. Thereafter, the samples were ashed in a covered crucible at 550°C in a furnace for 16 h to obtain a white residual ash. The ashes were subjected to an acid digestion process in an Erlen-flask with concentrated HNO_3 (E-Merck; 14 mL) until no brown fumes appeared. The digestion was continued over the sand bath with HClO_4 (12 mL) until the color of the solution became pale yellow to colorless. The solution was thereafter cooled and filtered through Whatman No. 1 filter paper. The filtrate was diluted with distilled water (50 mL) before being aspirated in the atomic absorption spectrophotometer for the determination of minerals. The analyses of Ca, Mg, Na, K, Mn, Fe, and Zn were performed by atomic absorption spectrophotometry. For Se, continuous flow hydride generator (CHEMITO) coupled with the atomic absorption spectrometer was used. Phosphorus content was analyzed by alkalimetric ammonium molybdophosphate method as described earlier (AOAC, 2002).

Chlorophyll-a concentration and sea surface temperature

The chlorophyll-a concentrations were derived from global 9-km monthly mean SeaWiFS (Sea Viewing Wide Field-of-view Sensor) data for the period from April to May 2008–2011 (<http://reason.gsfc.nasa.gov/OPS/Giovanni/ocean.seawifs.shtml>) to indicate the distribution of the photosynthetic pigment chlorophyll-a and expressed as mg/m^3 . Sea surface temperature (SST) was derived from global 9-km monthly mean MODIS (Moderate Resolution Imaging Spectroradiometer)–Aqua data for the period April to May 2008–2011 (<http://reason.gsfc.nasa.gov/OPS/Giovanni/ocean.seawifs.shtml>), which represents the temperature at the top 0.1 mm of water column (Chakraborty and Joseph, 2015).

Phytoplankton density and diatom concentration in water collected from the southwest coast of india

The planktonic diatoms were collected from the points of oyster collection site by towing a SEA-GEAR Model 900 phytoplankton net (SEA-GEAR Corp., Melbourne, FL, USA) for 20 min. Horizontal and vertical net tows were taken with a 30 cm diameter, 5 µm mesh phytoplankton net with a standard length: width ratio of 3:1. The plankton samples were suspended in cold clean seawater and stored in polyethylene bottles after preservation with Lugol's iodine solution. After concentrating the sample by sedimentation method, a 1 mL sample was taken and examined with a Sedgwick rafter counting chamber under a compound microscope (Nikon Eclipse-E600; Nikon Corp., Tokyo, Japan) with different magnifications. Water samples were collected from the surface at the same time as or a few days prior to oyster sampling, then preserved in 4% neutralized formalin and used for qualitative analysis.

Statistical analyses

Statistical evaluation was carried out with the Statistical Program for Social Sciences Version 13.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated for all the studied variables. Analyses were carried out in triplicate, and the means of all parameters were examined for significance by analysis of variance (ANOVA). The Pearson correlation test was used to assess correlations between means. The level of significance for all analyses was $p \leq 0.05$.

Results

Morphometric characteristics of the edible oysters grown at the wild and cultured habitats

The present study established no significant differences ($p > 0.05$) in the condition indices between the edible oysters grown at the wild and cultured habitats by taking into account the methods of Walne (1976) and Booth (1983). The condition indices measured by the methods of Imai and Sakai (1961), Walne, and Booth were at their maxima in the *C. madrasensis* grown under the cultured growth condition during 2010 and 2011. Significant differences in meat yield were recorded between the edible oysters in the wild and cultured growth habitats, with higher values recorded in the cultured samples ($p < 0.05$). However, the meat yield of the edible oysters from the wild growth habitat showed that this morphometric index was at the highest during 2010 to 2011, whereas those grown at the cultured tract showed that the meat yield were at the highest during the period between 2008 and 2009.

Lipid content and fatty acid composition of wild and cultured oysters

The lipid content and fatty acid composition of *C. madrasensis* are shown in Table 1. The oysters collected from the wild habitats exhibited higher lipid content (6–7%) as compared to those grown at cultured ambience (5.4–5.9%).

The total SFAs were recorded at their minimum in the edible oysters collected under the cultured growth condition and at maximum in those grown at the wild ambience. Myristic (C14:0), palmitic (C16:0), and stearic acids (C18:0) were the most common SFAs. Significant annual variations were apparent between the cultured and wild oysters with regard to SFA contents during the experimental period spanning from 2008–2011 ($p < 0.05$; Table 1). Palmitic acid (C16:0) was the primary SFA found in the wild and cultured edible oysters, contributing 48–61% to the total SFA content in the wild and 51–63% to the total SFA content in the cultured samples.

Significantly higher MUFA content was observed in the cultured samples obtained during the study period ($p < 0.05$). Myristoleic (C14:1*n*-7), palmitoleic (C16:1*n*-7), and oleic acids (C18:1*n*-9) were the major MUFAs in the muscle tissue of *C. madrasensis*, whereas C18:1*n*-9 and C16:1*n*-7

Table 1. Lipid and fatty acid composition of *C. madrasensis* collected from two different growth conditions (wild and cultured) and years (2008–2012) from the southwest coast of India.

	Wild				Cultured			
	2008	2009	2010	2011	2008	2009	2010	2011
Lipid (%)	6.82 ± 0.23 ^a	6.07 ± 0.17 ^a	7.04 ± 0.38 ^a	6.70 ± 0.07 ^a	5.88 ± 0.01 ^a	5.94 ± 0.01 ^a	5.63 ± 0.01 ^a	5.43 ± 0.22 ^a
Saturated fatty acids								
C12:0	0.65 ± 0.02 ^{ab}	ND	0.14 ± 0.02 ^a	1.13 ± 0.08 ^b	0.98 ± 0.01 ^b	2.95 ± 0.03 ^c	0.2 ± 0.03 ^c	0.52 ± 0.06 ^c
C14:0	3.06 ± 0.24 ^a	3.99 ± 0.07 ^a	4.36 ± 0.09 ^a	2.84 ± 0.03 ^b	2.04 ± 0.32 ^c	3.07 ± 0.24 ^c	7.84 ± 0.37 ^c	6.56 ± 0.45 ^c
C15:0	0.56 ± 0.04 ^a	2.35 ± 0.10 ^b	0.92 ± 0.30 ^a	0.46 ± 0.02 ^a	0.54 ± 0.02 ^a	0.63 ± 0.01 ^c	1.28 ± 0.27 ^c	1.85 ± 0.53 ^c
C16:0	28.1 ± 0.73 ^a	30.5 ± 0.54 ^a	19.9 ± 0.10 ^b	27.7 ± 0.37 ^a	27.5 ± 0.50 ^a	32.2 ± 1.12 ^a	23.7 ± 0.28 ^b	25.1 ± 0.10 ^a
C17:0	0.22 ± 0.02 ^a	ND	6.95 ± 0.08 ^b	2.46 ± 0.04 ^c	0.35 ± 0.06 ^a	0.14 ± 0.04 ^a	3.83 ± 0.51 ^b	2.34 ± 0.75 ^c
C18:0	9.67 ± 0.36 ^a	8.52 ± 0.27 ^a	5.81 ± 0.07 ^b	6.62 ± 0.26 ^b	12.4 ± 1.22 ^a	8.56 ± 0.92 ^a	7.63 ± 0.37 ^{ab}	8.57 ± 0.63 ^a
C20:0	0.47 ± 0.02 ^a	0.40 ± 0.01 ^a	2.12 ± 0.70 ^b	1.47 ± 0.17 ^b	0.83 ± 0.12 ^a	0.28 ± 0.01 ^a	0.28 ± 0.01 ^a	0.31 ± 0.02 ^a
C22:0	0.43 ± 0.01 ^a	ND	0.43 ± 0.02 ^a	0.38 ± 0.03 ^a	ND	0.17 ± 0.01 ^a	0.2 ± 0.01 ^a	0.22 ± 0.02 ^a
C24:0	3.73 ± 0.08 ^a	3.75 ± 0.09 ^a	0.04 ± 0.01 ^b	1.75 ± 0.07 ^c	2.24 ± 0.15 ^c	2.73 ± 0.11 ^c	0.85 ± 0.56 ^d	2.30 ± 0.93 ^c
ΣSFA	47 ± 0.57 ^{ac}	49.5 ± 0.37 ^a	40.7 ± 0.60 ^b	44.8 ± 0.51 ^c	46.9 ± 0.57 ^{ac}	50.8 ± 0.57 ^a	45.9 ± 0.67 ^c	47.8 ± 0.98 ^a
Monounsaturated fatty acids								
C14:1 n-7	ND	ND	0.54 ± 0.02 ^a	ND	ND	ND	1.20 ± 0.13 ^b	0.68 ± 0.02 ^a
C16:1 n-7	9.12 ± 0.06 ^a	9.32 ± 0.04 ^a	3.50 ± 0.20 ^b	5.72 ± 0.19 ^c	5.01 ± 0.04 ^c	4.88 ± 0.03 ^c	5.38 ± 0.35 ^c	6.14 ± 0.66 ^c
C18:1 n-7	ND	ND	ND	ND	ND	ND	ND	ND ± ND
C18:1 n-9	10.4 ± 0.19 ^a	11.0 ± 0.14 ^a	4.14 ± 0.20 ^b	5.96 ± 0.16 ^{bd}	17.5 ± 0.14 ^c	17.0 ± 0.10 ^c	5.79 ± 0.43 ^d	7.96 ± 0.14 ^d
C20:1 n-9	ND	0.26 ± 0.01 ^a	0.43 ± 0.08 ^a	0.93 ± 0.12 ^a	1.04 ± 0.01 ^a	0.97 ± 0.02 ^a	0.21 ± 0.01 ^a	0.25 ± 0.05 ^a
C22:1 n-9	ND	ND	4.58 ± 0.16 ^a	4.46 ± 0.01 ^a	ND	ND	6.35 ± 0.20 ^a	5.33 ± 0.11 ^a
C24:1	2.24 ± 0.45 ^a	0.80 ± 0.33 ^b	0.04 ± 0.01 ^c	1.23 ± 0.28 ^d	2.00 ± 0.01 ^d	1.95 ± 0.01 ^d	0.26 ± 0.06 ^b	0.59 ± 0.03 ^b
ΣMUFA	22 ± 0.02 ^a	21.4 ± 0.14 ^{ac}	13.2 ± 0.05 ^b	18.3 ± 0.37 ^c	25.5 ± 0.20 ^d	24.9 ± 0.15 ^d	19.2 ± 0.50 ^c	20.9 ± 0.51 ^{ac}
Polyunsaturated fatty acids								
C 18:2n-6	ND	ND	ND	ND	ND	ND	ND	ND
C 18:3n-6	ND	ND	ND	ND	ND	ND	ND	ND
C 18:3n-3	3.48 ± 0.06 ^a	3.69 ± 0.04 ^a	4.27 ± 0.34 ^a	3.81 ± 0.24 ^a	1.38 ± 0.09 ^b	1.07 ± 0.07 ^b	4.42 ± 0.10 ^a	3.88 ± 0.02 ^a
C 18:4n-3	1.84 ± 0.13 ^a	1.39 ± 0.10 ^a	1.85 ± 0.10 ^a	2.33 ± 0.47 ^a	2.12 ± 0.06 ^a	2.34 ± 0.05 ^a	1.28 ± 0.04 ^a	1.48 ± 0.03 ^a
C 18:4n-6	1.49 ± 0.09 ^a	1.78 ± 0.06 ^a	0.68 ± 0.32 ^a	0.80 ± 0.12 ^a	0.62 ± 0.08 ^a	0.88 ± 0.06 ^a	0.73 ± 0.11 ^a	1.01 ± 0.20 ^a
C 20:2n-6	ND	ND	ND	ND	2.08 ± 0.31 ^a	1.06 ± 0.23 ^a	ND	ND
C 20:3n-6	1.49 ± 0.09 ^{ab}	1.78 ± 0.06 ^a	1.88 ± 0.11 ^a	1.68 ± 0.13 ^{ab}	1.12 ± 0.07 ^{ab}	1.36 ± 0.05 ^{ab}	0.72 ± 0.19 ^b	1.21 ± 0.31 ^{ab}
C 20:4n-6	0.66 ± 0.02 ^a	0.63 ± 0.02 ^a	3.32 ± 0.04 ^b	2.83 ± 0.04 ^b	0.56 ± 0.03 ^a	0.68 ± 0.02 ^a	2.23 ± 0.06 ^b	1.96 ± 0.03 ^b
C 20:4n-3	3.63 ± 0.17 ^{ab}	4.19 ± 0.13 ^b	1.52 ± 0.09 ^c	1.98 ± 0.05 ^c	2.06 ± 0.02 ^{ac}	2.15 ± 0.01 ^{ac}	1.57 ± 0.23 ^{ac}	2.16 ± 0.39 ^{ac}
C 20:5n-3	7.67 ± 0.33 ^a	6.61 ± 0.25 ^a	9.38 ± 0.07 ^c	8.91 ± 0.09 ^c	6.65 ± 0.31 ^a	5.65 ± 0.23 ^a	6.76 ± 0.20 ^a	7.13 ± 0.42 ^a
C 22:5n-3	0.66 ± 0.05 ^a	0.63 ± 0.02 ^a	1.33 ± 0.08 ^a	1.52 ± 0.29 ^a	1.69 ± 0.22 ^a	0.97 ± 0.04 ^a	0.59 ± 0.02 ^a	0.62 ± 0.01 ^a
C 22:6n-3	7.01 ± 0.32 ^a	5.97 ± 0.24 ^a	11.5 ± 0.10 ^b	9.86 ± 0.80 ^b	6.61 ± 0.05 ^a	5.26 ± 0.29 ^a	6.48 ± 0.15 ^a	6.72 ± 0.33 ^a
ΣPUFA	27.9 ± 0.39 ^a	26.7 ± 0.29 ^a	35.8 ± 0.08 ^b	33.7 ± 0.62 ^b	24.9 ± 0.72 ^{ac}	21.4 ± 0.79 ^c	24.8 ± 0.78 ^{ac}	26.2 ± 1.62 ^a
Σn-3	24.3 ± 0.57 ^a	22.5 ± 0.43 ^{ac}	29.9 ± 0.25 ^b	28.4 ± 0.25 ^b	20.5 ± 1.09 ^{cd}	17.4 ± 0.81 ^d	21.1 ± 0.47 ^{cd}	22 ± 0.89 ^{cd}
Σn-6	3.64 ± 0.18 ^a	4.19 ± 0.13 ^a	5.88 ± 0.29 ^a	5.31 ± 0.11 ^a	4.38 ± 0.11 ^a	3.98 ± 0.08 ^a	3.68 ± 0.31 ^a	4.18 ± 0.68 ^a
ΣC ₁₈ PUFA	6.8 ± 0.01 ^a	6.9 ± 0.01 ^a	6.8 ± 0.08 ^a	7 ± 0.11 ^a	4.1 ± 0.26 ^a	4.3 ± 0.20 ^a	6.4 ± 0.06 ^a	6.4 ± 0.15 ^a
ΣC ₂₀ PUFA	13.4 ± 0.07 ^a	13.2 ± 0.05 ^a	16.1 ± 0.78 ^b	15.4 ± 0.11 ^b	12.5 ± 0.17 ^c	10.9 ± 0.12 ^c	11.3 ± 0.57 ^{cd}	12.5 ± 1.10 ^{cd}
n-3/n-6	6.7 ± 0.07 ^a	5.4 ± 0.05 ^a	5.1 ± 0.14 ^a	5.4 ± 0.04 ^a	4.7 ± 0.18 ^a	4.4 ± 0.13 ^a	5.7 ± 0.02 ^a	5.3 ± 0.04 ^a

(Continued)

Table 1. (Continued).

	Wild				Cultured			
	2008	2009	2010	2011	2008	2009	2010	2011
<i>n</i> -6/ <i>n</i> -3	0.15 ± 0.02 ^a	0.19 ± 0.01 ^a	0.20 ± 0.02 ^a	0.19 ± 0.02 ^a	0.21 ± 0.01 ^a	0.23 ± 0.03 ^a	0.17 ± 0.01 ^a	0.19 ± 0.02 ^a
ΣPUFA/ΣSFA	0.60 ± 0.01 ^a	0.54 ± 0.01 ^a	0.88 ± 0.09 ^b	0.75 ± 0.03 ^b	0.53 ± 0.02 ^a	0.42 ± 0.01 ^a	0.54 ± 0.02 ^a	0.55 ± 0.01 ^a
DHA/EPA	0.91 ± 0.04 ^a	0.90 ± 0.03 ^a	1.23 ± 0.11 ^a	1.11 ± 0.03 ^a	0.99 ± 0.03 ^a	0.93 ± 0.02 ^a	0.96 ± 0.03 ^a	0.94 ± 0.01 ^a
AI	0.82	0.97	0.77	0.77	0.73	1.03	1.26	1.10
IT	0.44	0.50	0.29	0.36	0.52	0.62	0.49	0.48
HH	0.96	0.83	1.40	1.08	1.16	0.87	0.83	0.89

ΣSFA: total saturated fatty acids; ΣMUFA: total monounsaturated fatty acids; ΣPUFA: total polyunsaturated fatty acids. Data presented as mean values of three samples (mean ± SD). These values do not total 100% because minor fatty acids are not reported. ND implies nondetectable (or fatty acids present below 0.05%). Differently shown letters (a–d) in the same row are statistically different ($p < 0.05$).

amounts were higher than others. The total MUFA in the edible oyster grown in the wild habitats ranged from 13 to 22%, and 19 to 26% in those under cultured background. The fatty acid C16:1*n*-7, a diatom marker, registered significantly higher values ($p < 0.05$) in the oysters grown under the wild ambiance during 2008 and 2009. However, during the period of 2010 and 2011, the *C. madrasensis* grown under cultured conditions recorded significantly higher amount of palmitoleic acid ($p < 0.05$) than those from the wild habitats.

The wild samples of *C. madrasensis* recorded significantly higher PUFA content (27–36%; $p < 0.05$) than those grown under cultured habitats (21–26%) during the study period (2008 through 2011). The total content of *n*-3 PUFAs (C20:5*n*-3, EPA and C22:6*n*-3, DHA) was recorded to be significantly higher ($p < 0.05$) in the edible oysters grown at their wild ambiance than those in the cultured conditions. Interestingly, *C. madrasensis* grown under the wild and cultured habitats recorded significantly higher C₁₈ and C₂₀ PUFAs during 2010 and 2011 than those collected in the preceding years (2008 and 2009; $p < 0.05$). The ratio of *n*-3/*n*-6 PUFA ranged from 5.1–6.7 in the wild samples and 4.4–5.7 in the cultured samples of *C. madrasensis*. No significant differences were observed in the *n*-3/*n*-6 PUFA and DHA/EPA ratios between the edible oysters grown under the wild and cultured habitats during the studied period ($p > 0.05$). The significantly higher Σ PUFA/ Σ SFA ratio ($p < 0.05$) recorded in the wild samples collected during 2010 and 2011 was predominantly contributed by the *n*-3 fatty acids, particularly C20:5*n*-3, C22:5*n*-3, and C22:6*n*-3.

Total cholesterol content

The cultured samples of *C. madrasensis* exhibited significantly higher cholesterol content (42–45 mg %) than wild samples (31–35 mg %; $p < 0.05$) irrespective of the collection period (Table 2).

Amino acid composition

Seventeen amino acids were identified and quantified in the wild and cultured samples of *C. madrasensis* (Table 3). The edible oysters collected in the wild samples exhibited significantly higher ($p < 0.05$) amount of total essential (Σ EAA) and nonessential (Σ NEAA) amino acid contents than those in the cultured samples obtained during the study period (2008 through 2011). The most abundant essential amino acid was found to be arginine in the wild (3.2–3.3 g/100 g protein) and cultured (~ 2.3 g/100 g protein) *C. madrasensis*, followed by leucine and lysine. Among nonessential amino acids, glutamic acid constitutes the major share followed by glycine in both the wild (~ 2.9 g/100 g protein) and cultured (~ 2.2 g/100 g protein) oysters. However, the content of glycine was found to be significantly higher in the wild oysters ($p < 0.05$) than in the cultured samples. The total sulfated amino acid content (Σ SAA) was significantly higher in the wild oysters as compared to the cultured samples of *C. madrasensis* ($p < 0.05$). The total amino acid content (Σ AA) was found to be significantly higher ($p < 0.05$) in the wild oysters (~ 20 g/100 g protein) than that in the cultured *C. madrasensis* (~ 13g/100 g protein).

Vitamin composition

The fat soluble vitamins A, D₃, E, K, and water soluble vitamin C in the edible oyster are recorded in Table 2. The cultured samples exhibited significantly higher ($p < 0.05$) content of cholecalciferol during the studied periods. The levels of α -tocopherol (Vitamin E), a vitamin with antioxidant properties, showed no significant difference ($p > 0.05$) among wild (0.18–0.24 IU) and cultured (0.15–0.19 IU) samples of *C. madrasensis*. Phylloquinone (K₁) and vitamin C registered significantly higher values ($p < 0.05$) in wild samples than cultured edible oyster.

Table 2. Biometric measurements, condition indices, meat yield (%), cholesterol, vitamins, and mineral compositions of wild and cultured *C. madrasensis* collected through 2008–2011.

	Wild				Cultured			
	2008	2009	2010	2011	2008	2009	2010	2011
Meat yield	11.51 ± 0.05 ^a	12.40 ± 0.14 ^a	12.16 ± 0.09 ^a	13.17 ± 0.11 ^{abc}	15.0 ± 0.05 ^{bc}	16.75 ± 0.11 ^b	12.98 ± 0.08 ^{bc}	14.20 ± 0.11 ^{bc}
Length (cm)	9.14 ± 0.12 ^a	9.94 ± 0.23 ^a	10.5 ± 0.12 ^a	11 ± 0.05 ^a	9.21 ± 0.11 ^a	10.25 ± 0.21 ^a	10.7 ± 0.31 ^a	11.4 ± 0.05 ^a
Thickness (cm)	3.20 ± 0.01 ^a	3.5 ± 0.02 ^a	3.90 ± 0.06 ^a	4.15 ± 0.09 ^a	3.30 ± 0.12 ^a	3.90 ± 0.01 ^a	3.80 ± 0.02 ^a	4.10 ± 0.06 ^a
Width (cm)	5.0 ± 0.01 ^a	5.1 ± 0.02 ^a	8.5 ± 0.06 ^b	9.0 ± 0.05 ^b	5.41 ± 0.03 ^a	4.86 ± 0.25 ^a	8.6 ± 0.07 ^b	8.9 ± 0.09 ^b
Condition indices								
Imai and Sakai	0.45 ± 0.01 ^a	0.47 ± 0.01 ^a	0.41 ± 0.01 ^a	0.42 ± 0.02 ^a	0.45 ± 0.03 ^a	0.52 ± 0.04 ^b	0.39 ± 0.02 ^c	0.40 ± 0.06 ^a
Booth	0.12 ± 0.01 ^a	0.12 ± 0.02 ^a	0.12 ± 0.01 ^a	0.13 ± 0.02 ^a	0.15 ± 0.03 ^a	0.17 ± 0.02 ^a	0.13 ± 0.02 ^a	0.14 ± 0.01 ^a
Walne	0.02 ± 0 ^a	0.03 ± 0 ^a	0.02 ± 0 ^a	0.03 ± 0.01 ^a	0.03 ± 0.00 ^a	0.04 ± 0.01 ^a	0.03 ± 0.01 ^a	0.03 ± 0.01 ^a
Fat soluble vitamins								
Retinol (A)/IU	5.26 ± 0.08 ^a	4.99 ± 0.06 ^a	5.35 ± 0.16 ^a	5.17 ± 0.06 ^a	4.00 ± 0.00 ^a	3.98 ± 0.00 ^a	4.26 ± 0.08 ^a	4.22 ± 0.02 ^a
Cholecalciferol (D ₃)/IU	441.9 ± 4.43 ^a	456.0 ± 3.32 ^b	439.9 ± 1.33 ^{ad}	434.0 ± 2.88 ^d	456.1 ± 7.74 ^b	480.7 ± 3.81 ^c	459.2 ± 0.8 ^b	445.4 ± 4.2 ^a
Tocopherol (E)/IU	0.24 ± 0.01 ^a	0.18 ± 0.01 ^a	0.20 ± 0.00 ^a	0.22 ± 0.01 ^a	0.15 ± 0.00 ^a	0.18 ± 0.00 ^a	0.15 ± 0.02 ^a	0.19 ± 0.03 ^a
Phylloquinone (K ₁) (µg/100 g)	1.34 ± 0.01 ^a	1.40 ± 0.01 ^b	1.50 ± 0.01 ^c	1.52 ± 0.03 ^c	0.91 ± 0.01 ^d	0.86 ± 0.01 ^e	0.95 ± 0.01 ^d	0.96 ± 0.02 ^d
Water soluble vitamins								
Ascorbic acid (C)/IU	10.3 ± 0.03 ^a	10.4 ± 0.02 ^a	9.76 ± 0.30 ^b	10.0 ± 0.08 ^a	9.52 ± 0.10 ^c	9.85 ± 0.07 ^{ab}	9.78 ± 0.03 ^b	9.66 ± 0.11 ^d
Moisture (%)	83.7 ± 0.34 ^a	84.8 ± 0.26 ^a	81.2 ± 0.67 ^a	80.8 ± 1.05 ^a	82.5 ± 0.25 ^a	83.3 ± 0.18 ^a	82.0 ± 1.27 ^a	78.5 ± 3.08 ^a
Cholesterol (mg %)	30.6 ± 0.38 ^a	31.9 ± 0.29 ^a	34.5 ± 0.96 ^a	33.7 ± 0.08 ^a	41.6 ± 0.92 ^b	44.5 ± 0.69 ^b	43.3 ± 0.72 ^b	44.3 ± 0.51 ^b
Macronutrients (mg/100 g wet sample)								
Na	192.3 ± 1.26 ^{ac}	196.4 ± 0.95 ^a	185.6 ± 3.93 ^b	188.5 ± 0.20 ^{bc}	144.0 ± 2.69 ^{de}	135.4 ± 2.02 ^f	146.5 ± 4.73 ^e	142.0 ± 0.42 ^d
K	270.1 ± 0.61 ^{ab}	272.0 ± 0.46 ^a	265.4 ± 4.48 ^b	271.9 ± 3.82 ^a	511.7 ± 2.22 ^c	504.6 ± 1.66 ^d	515.4 ± 2.21 ^c	511.9 ± 0.70 ^c
Ca	168.1 ± 1.61 ^a	173.2 ± 1.21 ^{ac}	174.7 ± 1.36 ^{bc}	158.8 ± 1.23 ^d	123.5 ± 1.53 ^e	118.6 ± 1.15 ^e	120.8 ± 0.16 ^e	122.4 ± 1.72 ^e
P	319.7 ± 2.72 ^a	328.4 ± 2.04 ^{ab}	319.1 ± 1.53 ^b	310.6 ± 2.62 ^c	518.4 ± 2.54 ^d	526.5 ± 1.91 ^d	530.2 ± 4.97 ^d	520.9 ± 3.63 ^d
Minonutrients (mg/100 g wet sample)								
Fe	3.37 ± 0.08 ^a	3.1 ± 0.06 ^b	3.14 ± 0.01 ^a	3.20 ± 0.05 ^c	4.26 ± 0.04 ^d	4.12 ± 0.03 ^e	4.23 ± 0.01 ^e	4.33 ± 0.10 ^d
Mn	ND	ND	ND	0.44 ± 0.13	ND	ND	ND	0.43 ± 0.05
Zn	3.56 ± 0.01 ^a	3.60 ± 0.01 ^a	3.66 ± 0.16 ^a	3.32 ± 0.26 ^a	4.43 ± 0.07 ^b	4.20 ± 0.05 ^b	4.48 ± 0.14 ^b	4.30 ± 0.08 ^b
Se	0.01 ± 0.00 ^a	0.02 ± 0.00 ^a	0.04 ± 0.00 ^a	0.05 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.02 ± 0.01 ^a
Na/K	0.6016	0.598	0.5815	0.607	0.2572	0.2764	0.2725	0.6016
Ca + P	487.82	501.64	493.9	469.53	645.2	651.1	643.46	487.82
Σ micronutrients	6.945	6.7312	6.8545	7.0197	8.3637	8.7626	9.1	6.945

All samples were analyzed in triplicate (n = 3) from pooled samples (30 numbers) and expressed as mean ± SD. Means followed by the same letter within the same row are not significantly different, and differently shown letters (a-f) in the same row are statistically different (p < 0.05). ND: non-detectable.

Table 3. Amino acid composition (g/100 g protein) of wild and cultured *C. madrasensis*.

	Wild				Cultured			
	2008	2009	2010	2011	2008	2009	2010	2011
Essential amino acids (E)								
Histidine (His)* (1.9 mg/100 g)	0.49 ± 0.01 ^a	0.50 ± 0.01 ^a	0.55 ± 0.01 ^a	0.54 ± 0.02 ^a	0.40 ± 0.03 ^a	0.38 ± 0.02 ^a	0.38 ± 0.07 ^a	0.39 ± 0.03 ^a
Arginine (Arg)*	3.19 ± 0.02 ^a	3.26 ± 0.01 ^a	3.31 ± 0.03 ^a	3.27 ± 0.02 ^a	2.26 ± 0.02 ^a	2.28 ± 0.05 ^a	2.30 ± 0.04 ^a	2.32 ± 0.02 ^a
Threonine (Thr)* (3.4 mg/100 g)	0.79 ± 0.01 ^a	0.83 ± 0.3 ^a	0.77 ± 0.09 ^a	0.73 ± 0.04 ^a	0.58 ± 0.03 ^a	0.57 ± 0.06 ^a	0.57 ± 0.02 ^a	0.53 ± 0.03 ^a
Valine (Val)* (3.5 mg/100 g)	0.61 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.08 ^a	0.63 ± 0.01 ^a	0.50 ± 0.02 ^a	0.50 ± 0.04 ^a	0.50 ± 0.03 ^a	0.50 ± 0.05 ^a
Methionine (Met)*	0.54 ± 0.01 ^a	0.48 ± 0.01 ^a	0.48 ± 0.02 ^a	0.48 ± 0.01 ^a	0.27 ± 0.04 ^a	0.25 ± 0.05 ^a	0.26 ± 0.09 ^a	0.28 ± 0.02 ^a
Isoleucine (Ileu)* (2.8 mg/100 g)	0.64 ± 0.01 ^a	0.61 ± 0.04 ^a	0.62 ± 0.03 ^a	0.64 ± 0.01 ^a	0.38 ± 0.02 ^a	0.36 ± 0.01 ^a	0.39 ± 0.01 ^a	0.38 ± 0.02 ^a
Leucine (Leu)* (6.6 mg/100 g)	1.57 ± 0.08 ^a	1.54 ± 0.11 ^a	1.56 ± 0.01 ^a	1.60 ± 0.03 ^a	1.10 ± 0.01 ^a	1.07 ± 0.04 ^a	1.06 ± 0.04 ^a	1.08 ± 0.02 ^a
Phenylalanine (Phe)*	0.88 ± 0.06 ^a	0.87 ± 0.02 ^a	0.87 ± 0.09 ^a	0.89 ± 0.01 ^a	0.55 ± 0.03 ^a	0.53 ± 0.04 ^a	0.51 ± 0.02 ^a	0.51 ± 0.01 ^a
Lysine (Lys)* (5.8 mg/100 g)	1.36 ± 0.04 ^a	1.37 ± 0.02 ^a	1.46 ± 0.02 ^a	1.45 ± 0.02 ^a	0.87 ± 0.03 ^a	0.90 ± 0.02 ^a	0.90 ± 0.04 ^a	0.89 ± 0.01 ^a
Nonessential amino acids (NE)								
Glutamic acid (Glu)**	2.86 ± 0.01 ^a	2.89 ± 0.04 ^a	2.91 ± 0.02 ^a	2.93 ± 0.09 ^a	2.23 ± 0.01 ^a	2.20 ± 0.07 ^a	2.20 ± 0.11 ^a	2.18 ± 0.04 ^a
Serine (Ser)**	1.58 ± 0.02 ^a	1.49 ± 0.01 ^a	1.50 ± 0.04 ^a	1.62 ± 0.09 ^a	0.87 ± 0.01 ^a	0.90 ± 0.01 ^a	0.92 ± 0.07 ^a	0.92 ± 0.04 ^a
Glycine (Gly)**	2.75 ± 0.02 ^a	2.68 ± 0.01 ^a	2.73 ± 0.07 ^a	2.72 ± 0.11 ^a	1.16 ± 0.06 ^b	1.21 ± 0.05 ^b	1.26 ± 0.04 ^b	1.29 ± 0.05 ^b
Alanine (Ala)**	1.37 ± 0.04 ^a	1.42 ± 0.07 ^a	1.42 ± 0.04 ^a	1.40 ± 0.03 ^a	1.09 ± 0.04 ^a	1.09 ± 0.07 ^a	1.06 ± 0.04 ^a	1.00 ± 0.05 ^a
Proline (Pro)**	0.80 ± 0.01 ^a	0.84 ± 0.08 ^a	0.85 ± 0.02 ^a	0.79 ± 0.04 ^a	0.69 ± 0.01 ^a	0.72 ± 0.07 ^a	0.65 ± 0.01 ^a	0.64 ± 0.02 ^a
Tyrosine (Tyr)**	0.44 ± 0.07 ^a	0.43 ± 0.04 ^a	0.44 ± 0.02 ^a	0.44 ± 0.02 ^a	0.48 ± 0.01 ^a	0.42 ± 0.01 ^a	0.47 ± 0.04 ^a	0.46 ± 0.02 ^a
Cysteine (Cys)**	0.44 ± 0.06 ^a	0.42 ± 0.05 ^a	0.40 ± 0.06 ^a	0.38 ± 0.02 ^a	0.08 ± 0.04 ^b	0.06 ± 0.05 ^b	0.06 ± 0.04 ^b	0.07 ± 0.07 ^b
ΣAA	20.31 ± 0.05 ^a	20.28 ± 0.04 ^a	20.52 ± 0.37 ^a	20.51 ± 0.25 ^a	13.51 ± 0.01 ^b	13.47 ± 0.01 ^b	13.49 ± 0.13 ^b	13.44 ± 0.12 ^b
ΣEAA	10.07 ± 1.23 ^a	10.11 ± 1.01 ^a	10.27 ± 2.05 ^a	10.23 ± 1.11 ^a	6.91 ± 0.32 ^b	6.87 ± 0.41 ^b	6.87 ± 0.11 ^b	6.56 ± 0.40 ^b
ΣNEAA	10.24 ± 1.11 ^a	10.17 ± 0.01 ^a	10.25 ± 0.01 ^a	10.28 ± 0.01 ^a	6.6 ± 0.33 ^b	6.6 ± 0.14 ^b	6.62 ± 0.41 ^b	6.60 ± 0.23 ^b
EAA/NEAA	0.98 ± 0.04 ^a	0.99 ± 0.06 ^a	1.0 ± 0.01 ^a	1.0 ± 0.05 ^a	1.05 ± 0.03 ^a	1.04 ± 0.02 ^a	1.04 ± 0.02 ^a	1.05 ± 0.02 ^a
ΣArAA	1.81 ± 0.02 ^a	1.80 ± 0.03 ^a	1.86 ± 0.05 ^a	1.87 ± 0.04 ^a	1.43 ± 0.01 ^a	1.33 ± 0.02 ^a	1.36 ± 0.03 ^a	1.36 ± 0.01 ^a
ΣSAA	0.98 ± 0.01 ^a	0.90 ± 0.02 ^a	0.88 ± 0.03 ^a	0.86 ± 0.01 ^a	0.35 ± 0.01 ^b	0.31 ± 0.02 ^b	0.32 ± 0.01 ^b	0.35 ± 0.02 ^b
Leu/Ileu	2.45 ± 0.11 ^a	2.52 ± 0.21 ^a	2.52 ± 0.36 ^a	2.50 ± 0.12 ^a	2.89 ± 0.16 ^a	2.97 ± 0.11 ^a	2.72 ± 0.21 ^a	2.84 ± 0.22 ^a
ΣEAA:ΣAA	0.50 ± 0.01 ^a	0.50 ± 0.02 ^a	0.50 ± 0.02 ^a	0.50 ± 0.02 ^a	0.51 ± 0.03 ^a	0.51 ± 0.01 ^a	0.51 ± 0.03 ^a	0.51 ± 0.05 ^a
ΣNEAA:ΣAA	0.50 ± 0.02 ^a	0.50 ± 0.01 ^a	0.50 ± 0.03 ^a	0.50 ± 0.02 ^a	0.49 ± 0.02 ^a	0.49 ± 0.02 ^a	0.49 ± 0.02 ^a	0.49 ± 0.01 ^a
Cys: ΣSAA	0.45 ± 0.02 ^a	0.47 ± 0.21 ^a	0.45 ± 0.05 ^a	0.44 ± 0.06 ^a	0.23 ± 0.01 ^b	0.19 ± 0.02 ^b	0.19 ± 0.01 ^b	0.20 ± 0.03 ^b

Reversed-phase binary gradient high performance liquid chromatograph (HPLC, Waters RP PICO:TAG amino acid analysis system), fitted with a column maintained at 38 ± 1°C in a column oven to be detected by their UV absorbance (λ_{max} 254 nm). The mobile phase eluents used were A and B. Eluent A comprises sodium acetate trihydrate (MeCOONa, 0.14 M, 940 mL, pH 6.4) containing TEA (Me₃N, 0.05%), mixed with CH₃CN (60 mL); and Eluent B used was acetonitrile:water (60:40, v/v). Data are means of triplicate analysis of pooled homogenates. Note: Tryptophan was not determined. *Essential amino acids for humans. ** Non-essential amino acids. Differently shown letters (a-b) in the same row are statistically different (p < 0.05).

Mineral composition

Table 2 shows the mineral composition of the cultured and wild oysters. The comparison between cultured and wild oysters revealed that there was significantly higher ($p < 0.05$) concentrations of Ca (159–175 mg/100 g) in the edible oysters collected from the wild habitats and K and P in the cultured oysters (K, 265–272 mg/100 g; P, 518–530 mg/100 g). In general, the present study revealed that *C. madrasensis* grown in the cultured growth condition exhibited higher micronutrient contents than those collected from the wild habitats. Selenium, the antioxidant element, was found to vary between 0.01–0.05 mg/100 g in the edible oyster collected from the wild habitat and 0.02–0.03 mg/100 g edible portion of the cultured samples.

Interannual variability in chlorophyll-a concentration and sea surface temperature (SST)

The variance in the spatial distribution of chlorophyll-a (<http://reason.gsfc.nasa.gov/OPS/Giovanni/ocean.seawifs.shtml>) during the study period (2008–2011) is recorded in Figures 2A–D. The mean chlorophyll-a concentration (mg/m^3) derived from the SeaWiFS data is recorded in Figure 3A. The chlorophyll-a concentration recorded maximum value in 2010 (4.76 mg/m^3) followed by 2011 (3.96 mg/m^3) and 2008 (1.46 mg/m^3). The SST data recorded during the premonsoon season (Figure 3B) indicated that high SST were observed throughout the study period ($> 29^\circ\text{C}$) with highest values recorded during 2011 (30.42°C).

Interannual variability in diatom population density along the southwest coast of india

Seasonal dynamics of phytoplankton biomass observed similar patterns during the study period. The phytoplankton density ranged from $7\text{--}20 \times 10^3$ cells/L. The sea water samples were found to be largely composed of diatoms, although annual variations were observed in their concentration, and their abundance was higher during 2008 and 2011 (16×10^3 cells/L) than the remainder of the study period. The predominant diatoms in the SW coast were found to be *Thalassiosira subtilis*, *Pleurosigma elongatum*, *Ditylum brightwellii*, *Nitzschia* sp., *Navicula* sp., *Isochrysis* sp., *Pleurosigma elongatum*, *Thalassiosira subtilis*, and *Biddulphia mobilensis*.

Discussion

The present study provided a detailed biochemical profile of edible oysters, *Crassostrea madrasensis*, collected from the wild and cultured growth habitats, from the prominent oyster bed along the southwestern coast of India. The samples of *C. madrasensis* were collected in May as there is a drop of salinity after May due to the monsoon. Therefore, this particular period has been chosen to collect both the wild and cultured samples of this target species. The periods of faster growth from April to May (premonsoon season) coincided with high chlorophyll-a levels. The chlorophyll-a peaks indicate an increase in primary production by May, associated with the increase in water temperature (Sasikumar et al., 2007; Purushan et al., 1983). Ojea et al. (2004) suggested that contents in the neutral lipids of the bivalve *Ruditapes decussatus* could be related to an increase in the chlorophyll-a concentration available in the diet. Dridi et al. (2007) also reported a positive correlation between total fatty acid values in Pacific oyster (*Crassostrea gigas*) and the chlorophyll-a in the food. Pazos et al. (2003) noted that environmental temperature influenced the levels of saturated and polyunsaturated fatty acids contents in the scallop (*Pecten maximus*). The commercial quality and physiological state of bivalve mollusks are adequately described by CI, a parameter of economic relevance reflecting the ecophysiological conditions and the health of oysters. CI and meat content of green oysters were registered to be affected by a variety of environmental and endogenous factors—viz., water temperature, salinity, food availability, and gametogenic cycle of animals (Okumus and Stirling, 1998). Positive correlations between the meat yield and chlorophyll-a levels in cultured

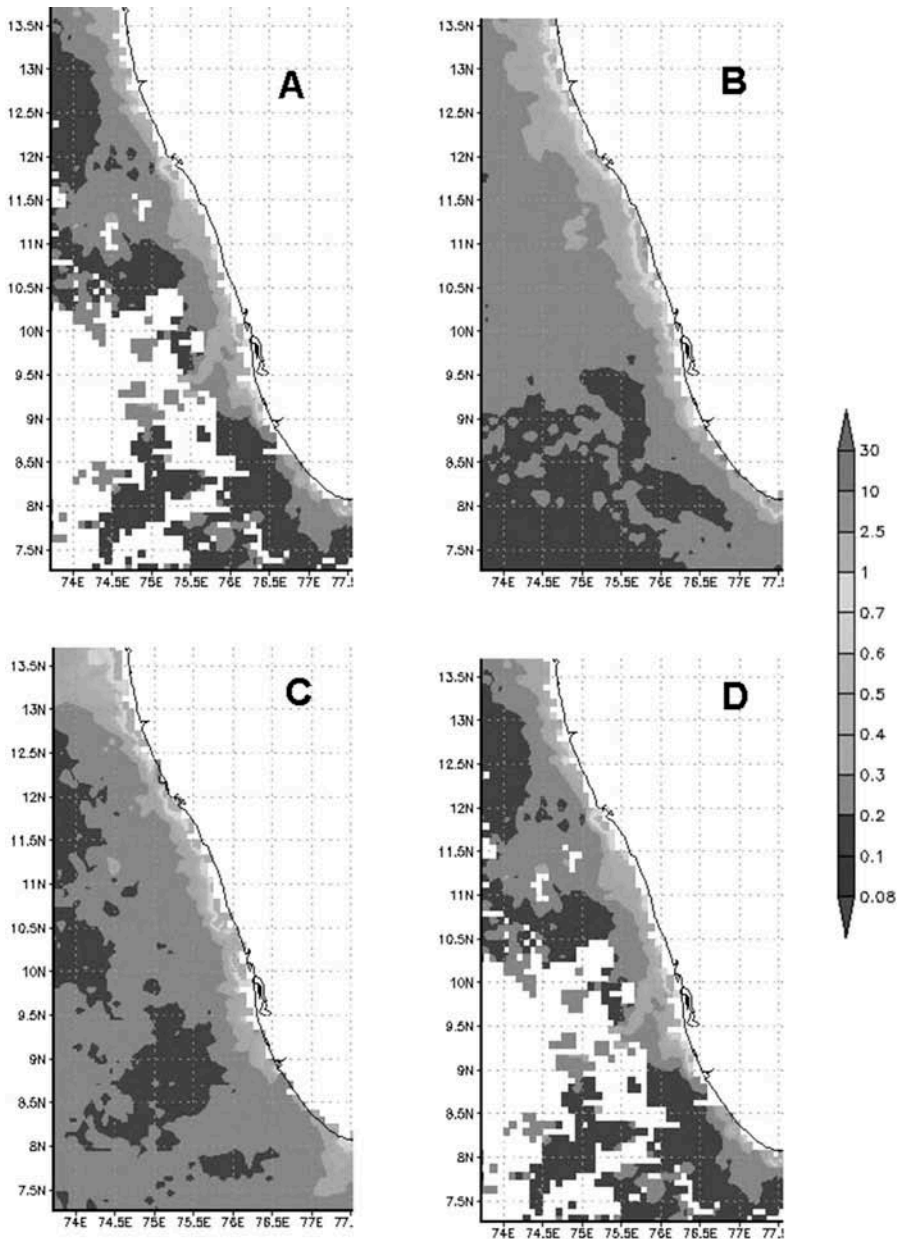


Figure 2. Satellite images of SeaWiFS chlorophyll-a concentration during premonsoon in the years 2008 (A); 2009 (B); 2010 (C); and 2011 (D).

oysters (Figure 4A, $r^2 = 0.88$) and negative correlation with wild oysters (Figure 4B, $r^2 = 0.13$) indicated that food availability and relatively high stocking densities are the important factors governing the increased growth of cultured oysters. The decreased growth rate of wild *C. madrasensis* may be due to some environmental factors such as disturbances in sites, competition for space, and slow water movement (Rajagopal et al., 2002). Maximum values of CI and meat yield in 2010 and 2011 were due to the seasonal blooms in Sattar Island waters in 2010 and 2011.

The higher relative percentage of diatoms (*Thalassiosira subtilis*, *Nitzschia seriata*, *Bacillaria paradoxa*, and *Biddulphia mobilensis*) and dinoflagellates in their diet (*Ceratium furca*, *C. tripos*,

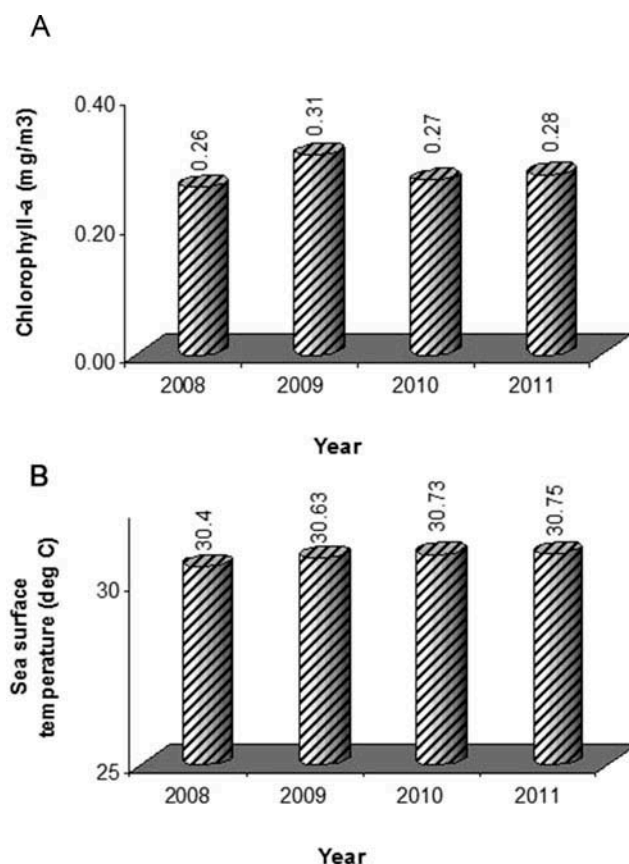


Figure 3. Seasonal variability of area-averaged time series of SeaWiFS mean chlorophyll-a concentration (mg/m^3) for the period of 4 years (2008–2011) during premonsoon (A) and variability of monthly mean sea surface temperature (SST) derived from global 9-km monthly mean MODIS-AQUA data for the 4 years (2008–2011) during premonsoon (B).

Gonyaulax spinifera, and *Peridinium biconicum*) with high lipid content appeared to contribute to the lipid pool of *C. madrasensis*. However, in the present study, the lipid content was lower as compared to an earlier study of Salaskar and Nayak (2011), who observed higher lipid content for *Crassostrea madrasensis* collected from Central west coast of India (12.3–14.1%). PUFAs are very important biochemical indicators of bivalves contributing to their nutritional quality. A wide range of variation in the fatty acid percentages in oysters has been detected, ranging from 24–36% for PUFA, 17–27% for MUFA, and 30–57% for SFAs. The fatty acid composition of marine bivalves depends on the biochemical and environmental conditions of seed development and environmental conditions, including the phytoplankton resources available (Fernández-Reiriz et al., 1989). The presence of readily available nonphytoplanktonic organic material in the cultured condition resulted in the accumulation of higher proportion of SFAs (Freites et al., 2002). The predominant MUFA, C18:1*n*-9, was significantly ($p < 0.05$) higher in cultured oysters than wild ones in all the years studied. The high amount of MUFA content in cultured oysters may be due to the high content of monoenoic fatty acids in the food of the cultured oysters. Significantly high levels of 16:1*n*-7 and 18:1*n*-9 were registered in phytoplanktons (diatoms and dinoflagellates), and this further supports the fact that foods ingested by bivalves when filter feeding is directly reflected in their fatty acid composition. As high level of PUFA is a characteristic of adaptation to aquatic habitats (Bulut et al., 2012), wild oysters are more adapted to aquatic habitats than cultured ones. Wild oysters recorded positive correlations between chlorophyll-a and PUFA composition (Figure 4C, $r^2 = 0.997$), and

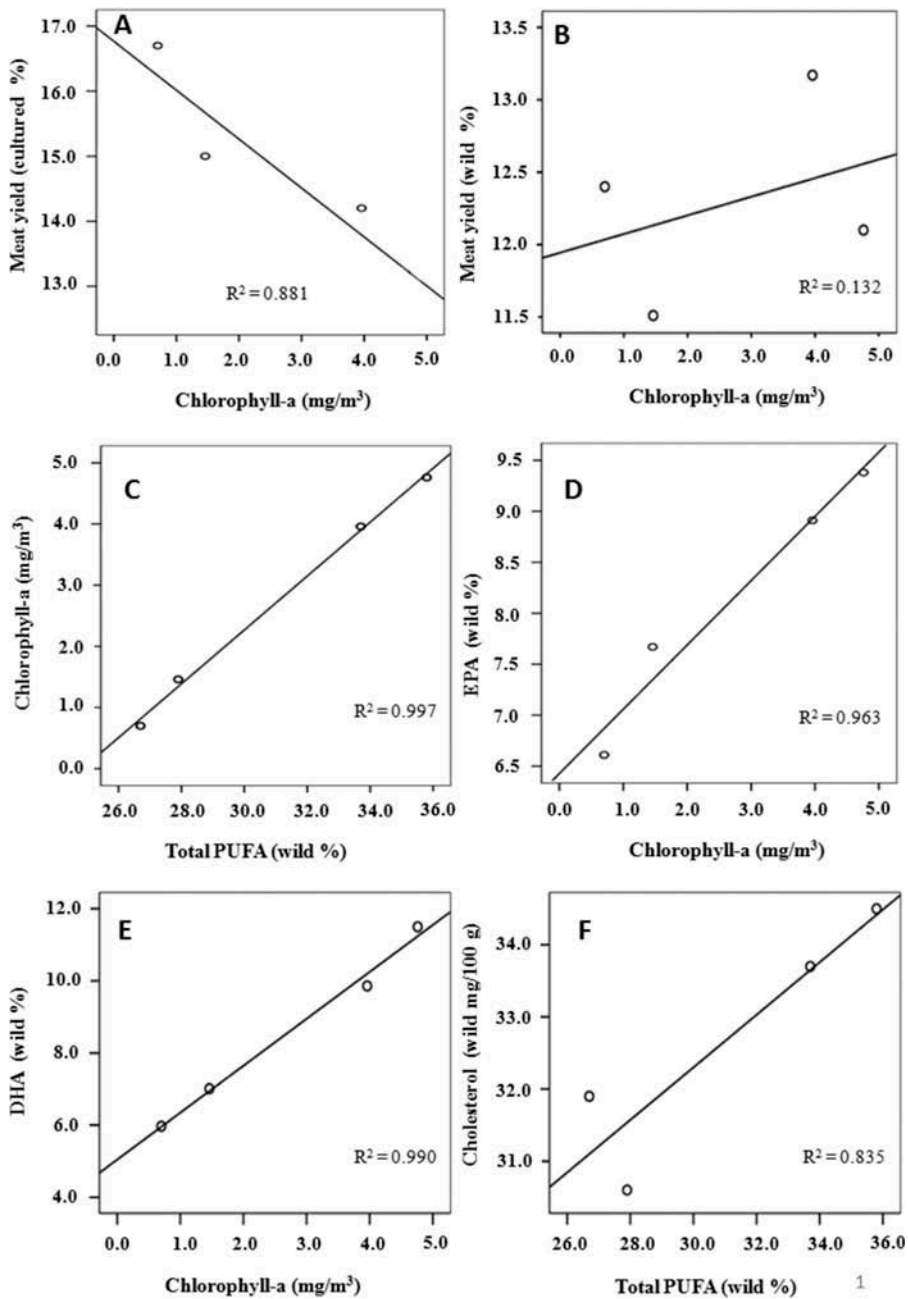


Figure 4. The correlation plots between cholesterol and total PUFA collected from wild condition (A); chlorophyll-a and EPA from wild condition (B); chlorophyll-a and DHA from wild condition (C); chlorophyll-a and meat yield from wild condition (D); chlorophyll-a and total PUFA from wild condition (E); and chlorophyll-a and meat yield from cultured condition (F).

maximum values of C₁₈ PUFA and C₂₀ PUFA in 2010 indicated that, besides gametogenic cycle influences, the blooming of different planktonic populations during the year may affect the lipid profile of those organisms, like bivalves, relying on plankton as the main food source (Albentosa et al., 1996).

Diatoms contain considerable amounts of 20:5*n*-3 (EPA) and 22:6*n*-3 (DHA), respectively, and hence DHA and EPA were the prominent PUFAs in wild and cultured oysters, followed by 20:4*n*-6 (AA) and 18:2*n*-6 (LA). There is a direct relation between the environmental temperature and the content of polyunsaturated fatty acids (Zibae-Nezhad et al., 2010), and similar results have been observed in wild and cultured oysters. Therefore, the ratio of DHA:EPA can be used as an indicator of the predominant phytoplankton class assimilated by the oysters. Ratios > 1 indicate a dominance of dinoflagellates in the diet, while ratios < 1 reflect a dominance of diatoms (Budge and Parrish 1998). A positive correlation was observed between EPA, DHA, and chlorophyll-*a* concentration in wild samples (Figure 4D, $r^2 = 0.96$; Figure 4E, $r^2 = 0.99$). Various algal species including dinoflagellates, cryptomonads, and certain thraustochytrids as well as zooplankton are known to contain elevated levels of DHA (Murphy et al., 2002). The UK Department of Health recommends an ideal ratio of *n*-6/*n*-3 of 4.0 at maximum (Cardiovascular Review Group, 1994), and values higher than 4.0 are considered harmful to health and may promote cardiovascular diseases. In the present study, the *n*-6/*n*-3 ratio (Table 1) was low, which is common for oysters. Linehan et al. (1999) found an *n*-6/*n*-3 ratio of 0.17 (winter) and 0.23 (summer), in *Crassostrea gigas*. Similarly, Liraa et al. (2013) observed low *n*-6/*n*-3 ratio of 0.16 and 0.13 in winter and summer, respectively, for *Crassostrea rhizophorae* collected from Brazil.

Among *n*-6 PUFAs, 20:4*n*-6, 18:2*n*-6, and 20:3*n*-6 were the dominant fatty acids, and the total content of *n*-6 PUFAs is presumably associated with a high 18:2*n*-6 intake probably contributed to by a microbial diet (Abad et al., 1995).

Diets that are high in MUFAs and PUFAs are associated with reduced risk of cardiovascular disease and atherogenesis (Andrade et al., 2012; Chakraborty and Paulraj, 2009). The atherogenic and thrombogenic indices were found to be higher in the cultured samples compared with wild samples, which gives an indication of the attitude of a composite diet or a single food to protect from atherosclerosis and platelets aggregation. The higher *n*-3 fatty acid content and consequently the higher *n*-3/*n*-6 fatty acid ratio in the wild samples apparently contributed to lower atherogenic and thrombogenic indices. It has been reported that due to the antiatherogenic and antithrombogenic properties, the *n*-3 PUFAs play a major role to protect human beings from atherosclerosis and platelet aggregation (Barrento et al., 2010). The ideal HH ratio noted in the oysters also contributed toward its qualities to be judged as desirable from the consumer health perspective.

The cholesterol content of wild samples showed very good positive correlation with PUFA content (Figure 4F, $r^2 = 0.83$). Cholesterol generally ranges from around 2–90% of total sterols in various mollusks. Plankton is the predominant diet, which contains various sterols, and they can be incorporated into its tissues, and some sterols—*viz.*, cholesterol can be synthesized from plankton cholesterol precursors (McLean and Bulling, 2005). The wild samples were found to possess higher essential amino acids than cultured samples, although the difference appeared to be insignificant and present in quantities required for balanced nutrition. The richness of amino acids in wild oysters has also been related to the maximum ripeness (Dridi et al., 2007). The slight difference in the Σ EAA and Σ NEAA among wild and cultured oysters appeared to be due to the different environmental and nutritional conditions. This demonstrates the potential capability of *C. madrasensis*, growing in wild condition, to withstand salinity and adverse stress conditions during summer, because glycine or its conjugate (glycine betaine) was earlier reported to have unique osmolytic property (Eklund et al., 2005) and helps to protect the cells during summer against osmotic injury. These differences in cysteine, a nonessential sulfated amino acid, and total sulfated amino acid content (Σ SAA) in wild and cultured samples may be due to the proteolysis of amino acids that might have occurred to a lesser extent in the samples. In the present study, no significant differences were observed in the EAA/NEAA ratio among wild and cultured samples ($p > 0.05$). Any ratio of EAA/NEAA amino acids higher than 1.0 is considered to be excellent, and therefore it can be concluded that *C. madrasensis* are good sources of well-balanced proteins and high-quality protein source in respect to EAA/NEAA ratio.

All *trans* retinol underwent meager fluctuations reaching maxima in wild samples and minima in cultured samples. The values of *trans*-retinol content are within the limits to impart their beneficial effects. The levels of α -tocopherol, a vitamin with antioxidant properties, in oysters were low and showed minor fluctuations between wild and cultured samples ($p > 0.05$). Significant differences were apparent in phylloquinone (K_1) and cholecalciferol (D_3) content between wild and cultured samples ($p < 0.05$). Vitamin D precursors constitute a large proportion of the unsaponifiable fraction of mollusk lipids. As only plants synthesize α -tocopherol, phytoplanktons (diatoms and dinoflagellates) either direct or indirectly are the ultimate source of this compound for oysters. Among minor unsaponifiable components, HPLC analyses showed that oxygenated carotenoids, characterized by typical absorption spectra, were prevalent over the less polar, late-eluting, α -carotene and β -carotene. Due to the lack of suitable standard compounds, the early-eluting xanthophylls were not identified.

Minerals are nutrients that are conserved by the body and play a significant role in metabolism in the human body. The present study revealed that the cultured oysters registered higher content of K and P wild samples. Ca is an extremely important mineral that is often out of balance in persons with arteriosclerosis and thyroid diseases. The cultured samples recorded lower Na and Ca than wild samples and therefore rated high as a health food. The results in the present study agree with other studies, which reported oysters to be a good source of Ca, Zn, and Fe (Astorga España et al., 2007). Fe and Zn were predominant elements of the total trace mineral contents in cultured and wild oysters, respectively, and significantly higher concentrations ($p < 0.05$) of Fe and Zn were obtained in cultured oysters when compared to their wild counter parts. The concentration of trace minerals in oysters is influenced by a number of factors such as seasonal and biological differences (size, age, sex, and sexual maturity), food source, and environment (water chemistry, salinity, temperature, and contaminants; Lal, 1995). The variation of mineral content between wild and cultured oysters appeared to be due to the influence of several factors including food availability and differences in metal regulation (Astorga España et al., 2007). In addition, the differences in the mineral concentrations of the surrounding seawater could also influence their levels in bivalves (Paez-Osuna et al., 1995).

Conclusion

The present study provides insights into different biochemical and fatty acid variations of oysters collected under different growth conditions from the southwestern coast of India. Growth conditions play a vital role in physiological mechanisms of oysters guiding fatty acid metabolism. High levels of PUFA (21.7–36.4%) including *n*-3 PUFAs (12.5–23.2%), low levels of *n*-6 PUFA (maximum of 7% of total fatty acids), and relatively high *n*-3/*n*-6 PUFA ratio values characterized *C. madrasensis*. The ratios of essential/nonessential amino indicated that both oyster tissues are good sources of well-balanced proteins. The compositional nature of the oyster suggested its nutritional importance as a component of human diet.

Acknowledgments

The authors are thankful to the Indian Council of Agricultural Research, New Delhi for providing necessary facilities and encouragements to carry out this work. The authors thank the Director of the Central Marine Fisheries Research Institute for his guidance and support. Thanks are due to the Head of the Marine Biotechnology Division, Central Marine Fisheries Research Institute for facilitating the research activity.

Funding

Funding was provided by the Indian Council of Agricultural Research Outreach Activity-3 on “Nutrient Profiling of Fish as a Food for Health and Dietary Component.”

References

- Abad, M., Ruiz, C., Martinez, D., Mosquera, G., and Sanchez, J. L. 1995. Seasonal variations of lipids classes and fatty acids in flat oyster, *Ostrea edulis*, from San Cibrán (Galicia, Spain). *Comp. Biochem. Phys. C* 110: 109–118.
- Albentosa, M., Labarta, U., Fernandez-Reiriz, M. J., and Perez-Camacho, A. 1996. Fatty acid composition of *Ruditapes decussates* spat fed on different microalgae diets. *Comp. Biochem. Phys. A* 13: 113–119.
- Andrade, J., Marin, R., Apel, M. A., Raseira, M. B., and Henriques, A. 2012. Comparison of the fatty acid profiles of edible native fruit seeds from southern Brazil. *Int. J. Food Prop.* 15(4): 815–822.
- AOAC. 1995. Vitamin C (ascorbic acid) in vitamin preparations and juices: 2,6-Dichloroindophenol titrimetric method procedure No. 967.21. In: *Official Methods of Analysis of the Association of Official Analytical Chemists* (15th ed.). Arlington, VA: Author. Pp. 1058–1059.
- AOAC. 2002. Official method 995.11: Phosphorus (total) in foods. In: *Official Methods of Analysis of the Association of Official Analytical Chemists* (17th ed.), Gaithersburg, MD: Author.
- Astorga España, M. S., Rodriguez, E. M., and Diaz Romero, C. 2005. Sodium, K, Ca, Mg, Fe, Cu and Zn concentrations in mollusks from the Strait of Magellan (Chile): Their contribution to dietary intake. *Int. J. Food Sci. Nutr.* 56: 337–347.
- Astorga España, M. S., Rodriguez, E. M., and Diaz Romero, C. 2007. Comparison of mineral and trace element concentrations in two molluscs from the Strait of Magellan (Chile). *J. Food Comp. Anal.* 20: 273–279.
- Barrento, S., Marques, A., Teixeira, B., Mendes, R., Bandarra, N., Vaz-Pires, P., and Nunes, M. L. 2010. Chemical composition, cholesterol, fatty acid and amino acid in two populations of brown crab *Cancer pagurus*: Ecological and human health implications. *J. Food Comp. Anal.* 23: 716–725.
- Booth, J. D. 1983. Studies on twelve common bivalve larvae and bivalve spawning seasons. *New Zeal. J. Mar. Fresh.* 17: 231–265.
- Budge, S. M., and Parrish, C. C. 1998. Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. *Org. Geochem.* 29: 1547–1559.
- Bulut, S., Uysal, K., Cemek, M., Gok, V., Kuş, F., and Karaçali, M. 2012. Nutritional evaluation of seasonal changes in muscle fatty acid composition of common carp (*Cyprinus carpio*) in Karamik Lake, Turkey. *Int. J. Food Prop.* 15(4): 717–724.
- Cardiovascular Review Group. 1994. Nutritional Aspects of Cardiovascular Disease: Report of the Cardiovascular Review Group, Committee on Medical Aspects of Food Policy. London, UK: HMSO.
- Chakraborty, K., Deepu, J., and Chakkalakal, S. J. 2014. Seasonal and inter-annual lipid dynamics of spiny cheek grouper (*Epinephelus diacanthus*) in the southern coast of India. *J. Mar. Biol. Assoc. U.K.* 94(8): 1677–1686.
- Chakraborty, K., and Joseph, D. 2015. Inter-annual and seasonal dynamics of amino acid, mineral and vitamin composition of silver belly *Leiognathus splendens*. *J. Mar. Biol. Assoc. U.K.* 95: 817–828. doi: 10.1017/S0025315414001155
- Chakraborty, K., and Paulraj, R. 2009. Selective enrichment of n-3 polyunsaturated fatty acids with C₁₈–C₂₀ acyl chain length from sardine oil using *Pseudomonas fluorescens* MTCC 2421 lipase. *Food Chem.* 114: 142–150.
- Dridi, S., Salah Romdhane, M., and Elcfsi, M. H. 2007. Seasonal variation in weight and biochemical composition of the Pacific oyster, *Crassostrea gigas* in relation to the gametogenic cycle and environmental conditions of the Bizert lagoon, Tunisia. *Aquaculture* 263: 238–248.
- Eklund M., Bauer, E., Wamatu, J., and Mosenthin R. 2005. Potential nutritional and physiological functions of betaine in livestock. *Nutr. Res. Rev.* 18: 31–48.
- Fernández-Reiriz, M. J., Perez-Camacho, A., Ferreira, M. J., Blanco, J., Planas, M., and Campos, M. J. 1989. Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. *Aquaculture* 83: 17–37.
- Folch, J., Lees, M., and Stanley, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497–509.
- Freites, L., Labarta, U., and Fernandez-Reiriz, M. J. 2002. Evolution of fatty acid profiles of subtidal and rocky shore mussel seed (*Mytilus galloprovincialis*, Link.). Influence of environmental parameters. *J. Exp. Mar. Biol. Ecol.* 268: 185–204.
- Harikumar, G., and Rajendran, G. 2007. An over view of Kerala fisheries—with particular emphasis on aquaculture. Integrated Fisheries Project Souvenir. Pp. 1–19.
- Heinrikson, L., and Meredith, S. C. 1984. Amino acid analysis by reverse-phase high-performance liquid chromatography: Precolumn derivatization with phenylisothiocyanate. *Anal. Biochem.* 136: 65–74.
- Imai, T., and Sakai, S. 1961. Study on the breeding of the Japanese oyster, *Crassostrea gigas*. *Tohoku J. Agr. Res.* 12: 125–171.
- Lal, S. 1995. Macro and trace elements in fish and shellfish. In: *Fish and Fishery Products: Composition, Nutritive Properties and Stability*. Ruiter, A. (Ed.). Wallingford, UK: CAB International. Pp. 187–214.
- Linehan, G. L., O'Connor, T. P., and Burnell, G. 1999. Seasonal variation in the chemical composition and fatty acid profile of Pacific oysters (*Crassostrea gigas*). *Food Chem.* 64: 211–214.
- Liraa, G. M., Pascoal, J. C. M., Torres, E. A. F. S., Soares, R. A. M., Mendonça, S., Sampaio, G. R., Correia, M. S., Cabral, C. C. V. Q., Cabral Júnior, C. R., and López, A. M. Q. 2013. Influence of seasonality on the chemical composition of oysters (*Crassostrea rhizophorae*). *Food Chem.* 138(2–3): 786–790.

- Mallia, J. V., Muthiah, I. P., and Thomas, P. C. 2009. Performance of triploid edible oyster *Crassostrea madrasensis* (Preston): Gonad development and biochemical composition. *J. Mar. Biol. Assoc. India* 51(1): 81–86.
- McLean, C. H., and Bulling, K. R. 2005. Differences in lipid profile of New Zealand marine species over four seasons. *J. Food Lipids* 12: 313–326.
- Metcalf, L. D., Schimtz, A. A., and Pleka, J. R. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analyses. *Anal. Chem.* 38: 514–515.
- Murphy, J. K., Moone, D. B., Mann, J. N., Nichols, D. P., and Sinclair, J. A. 2002. Lipid, fatty acid and sterol composition of New Zealand green lipped mussel (*Perna canaliculus*) and Tasmanian blue mussel (*Mytilus edulis*). *Lipids* 37(6): 587–595.
- Nagabhushanam, R., and Bidarkar, D. S. 1978. Studies on seasonal changes in the biochemical constituents of the oyster *Crassostrea cucullata*. *Indian J. Fish.* 25: 156–164.
- Ojea, J., Pazos, A. J., Martinez, D., Novoa, S., Sanchez, J. L., and Abad, M. 2004. Seasonal variation in weight and biochemical composition of the tissues of *Ruditapes decussatus* in relation to the gametogenic cycle. *Aquaculture* 238: 451–468.
- Okumus I., and Stirling H. P. 1998. Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. *Aquaculture* 159: 249–261.
- Paez-Osuna, F., Frias-Espericueta, M. G., and Osuna-Lopez, J. I. 1995. Trace metal and concentrations in relation to season and gonadal maturation in the oyster *Crassostrea iridiscens*. *Mar. Environ. Res.* 40: 19–31.
- Pazos, A. J., Sánchez, J. L., Román, G., Pérez-Parallé, L. P., and Abad, M. T. 2003. Seasonal changes in lipid classes and fatty acid composition in the digestive gland of *Pecten maximus*. *Comp. Biochem. Phys. B* 134(2): 367–380.
- Purushan, K. S., Gopalan, U. K., and Rao, T. S. S. 1983. On setting of spat and growth of the edible oyster *Crassostrea madrasensis* (Preston) in Cochin backwater. *Proc. Symp. Coastal Aquaculture* 2: 444–450.
- Rajagopal, S., van der Velde, G., and Jenner, H. A. 2002. Effects of low level chlorination on zebra mussel, *Dreissena polymorpha*. *Water Res.* 36: 3029–3034.
- Salaskar, G. M., and Nayak, V. N. 2011. Nutritional quality of bivalves, *Crassostrea madrasensis* and *Perna viridis* in the Kali estuary, Karnataka, India. *Rec. Res. Sci. Technol.* 3(4): 6–11.
- Salo-Vaananen, P., Mattila, P., Lehtikoinen, K., Salmela-Molsa, E., and Piironen V. 2000. Simultaneous HPLC analysis of fat-soluble vitamins in selected animal products after small-scale extraction. *J. Agric. Food Chem.* 71: S535–S543.
- Santos-Silva, J., Bessa, R. J. B., and Santos-Silva, F. 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livest. Prod. Sci.* 77: 187–194.
- Sasikumar, G., Krishnakumar, P. K., Thomas, S., Sampathkumar, G., Nagaraja, D., and Bhat, G. S. 2007. Influence of environmental factors on growth rate of *Crassostrea madrasensis* (Preston) in suspended culture. *Asian Fish. Sci.* 20: 241–255.
- Taylor, A. G., and Savage, C. 2006. Fatty acid composition of New Zealand green-lipped mussels, *Perna canaliculus*: Implications for harvesting for *n-3* extracts. *Aquaculture* 261: 430–439.
- Ulbricht, T. L. V., and Southgate, D. A. T. 1991. Coronary heart disease: Seven dietary factors. *Lancet* 338: 985–992.
- Walne, P. R. 1976. Experiments on the culture in the sea of the butterfish *Venerupis decussata* L. *Aquaculture* 8: 371–381.
- Wanasundara, U. N., and Shahidi, F. 1999. Concentration of omega-3 polyunsaturated fatty acids of seal blubber oil by urea complexation: Optimization of reaction conditions. *Food Chem.* 65: 41–49.
- Zibae-Nezhad, M. J., Khosravi, M., Akbari, S., Bani-Asadi, N., and Golboostan, E. 2010. Omega-3 fatty acid composition of Persian Gulf fishes. *Int. J. Food Prop.* 13(3): 574–579.